CHAPTER 4

Growth of Ectomycorrhizal Fungi around Seeds and Roots

G. D. BOWEN and C. THEODOROU

I. Introduction

Study of the growth of ectomycorrhizal fungi around seeds and roots is highly relevant to proposed inoculation programs both in the technology of application and in management practices likely to lead to ready establishment of the inoculum. It may be necessary to select mycorrhizal fungi not only on their ability to give a desired tree response but also on their ability to colonize the root readily in a range of conditions and persist from season to season. Apart from this, however, growth of the fungus on the root surface has significant roles in disease resistance,

in spread of inoculum from tree to tree and along a root (Robertson, 1954; Wilcox, 1968), and in nutrient uptake in some cases, even without infection. Thus Neumann (1959) reported growth stimulation of *Eucalyptus camaldulensis* by the mycorrhizal fungus *Pisolithus tinctorius* in the absence of infection, and Lamb and Richards (1971a) have confirmed this with mycorrhizal fungi on *Pinus radiata* and *P. elliotii* var. *elliottii*. In addition leachates of soil in which mycorrhizal pines are growing can induce forking of short roots (Levisohn, 1960), and this could be due to fungal exudates or to exudates of the mycorrhizal association. Such observations as those above are consistent with findings (Bowen and Rovira, 1969) that noninfecting rhizosphere organisms can affect root morphology, physiology, and ion uptake.

Growth of hyphae into soils away from the root will be examined in Chapter 5 because of its importance in solute uptake from soils. In this Chapter the establishment phases of the fungus on the root will be examined, since this is critical to the success of inoculation programs. Microflora on the seed often have to face extreme, fluctuating environmental conditions (especially in direct seeding in the field) because of their proximity to the soil surface. The seed surface is also a site of great susceptibility to microbial competition since seed diffusates provide a ready nutrient source for many microorganisms. Later, colonization of the emerging root allows movement away from the variable surface conditions, and the somewhat selective environment of the rhizosphere (Rovira, 1965) may reduce competition and antagonism from soil microorganisms to some extent. However it is not until infection occurs in the much more selective and protected environment within the root that some security of tenure is achieved by the fungus.

The specialized behavior of mycorrhizal fungi, e.g., inability to use complex polysaccharides as sources of carbon and energy, their frequent requirements for various growth factors, and inhibition by humus extracts, identifies them as typical rhizosphere fungi, despite the lack of general study of a possible saprophytic behavior in the absence of the host and the ability of some to decompose litter. Many studies have been performed on requirements of mycorrhizal fungi in pure culture (see Harley, 1969) but these will be discussed here only as they relate to growth of fungi in the rhizosphere. Some of the laboratory culture studies on mycorrhizal fungi have only limited relevance to fungus growth in a natural environment, and, similarly, some studies on colonization of roots in simplified defined situations (e.g., Theodorou and Bowen, 1969) have limitations in relating them to much more complex natural situations. However, studies on laboratory media and the simplified systems we describe are useful steps in understanding growth on roots and in suggesting hypotheses, for experimental testing, on colonization in the field.

Such colonization studies are relevant also for noninfecting rhizosphere microorganisms and plant pathogens in their preinfection phase.

II. Survival and Germination of Propagules

Robertson (1954) demonstrated that wind-dispersed basidiospores brought about mycorrhizal formation in previously uninfected soils. By filtering air entering a plant growth room, Marx and Bryan (1969) eliminated casual mycorrhizal infection on *P. echinata,* but outside such rooms 90% infection occurred in trees grown in fumigated soils without inoculation. It is well known that some fungal spores can be distributed over very large distances by wind but no detailed studies have been performed on mycorrhizal fungi; two of the main problems in such studies are the detection of the organisms in small quantities and the unambiguous identification of them. Wind dispersal of spores is the most likely reason for Bowen's observations (1963) that mycorrhizal fungi for *P. radiata,* an introduced species to natural *Eucalyptus* areas, were almost invariably found in soils up to 2 miles from established pine forests but beyond that they were frequently absent. The distance over which spores are carried *in sufficient quantity* to inoculate trees will depend largely on the fungus, the prevailing wind direction at the time of fruiting, and factors such as temperature, humidity, and resistance of the spores to desiccation. There are good grounds for the assumption that mycorrhizal fungi are readily available to seedlings of *indigenous* tree species. However, even close to existing pine forests, Theodorou and Bowen (1970) found naturally occurring mycorrhizal fungi to be erratic in distribution and inoculation to be beneficial to pine growth.

Few studies have been carried out on the persistence of mycorrhizal fungi in the absence of their host plant. Because of the ready wind dispersal of spores (Robertson, 1954) there is some doubt about Wilde's conclusion (1946) that, once introduced to soil, mycorrhizal fungi will survive for considerable periods in the absence of the host. Theodorou and Bowen (1971b) demonstrated the ability of some mycorrhizal fungi to grow in the rhizosphere of nonhost plants and this would aid persistence in the absence of a host.

A. Survival of Propagules

A number of studies have shown that while mycelial forms of inoculum can survive successfully after inoculation into sterile soils (e.g., Theodorou, 1967) and in natural soils under ideal conditions, they frequently fail or establish poorly under other field conditions, e.g., where soil dries out or where biological antagonisms occur (Theodorou, 1971). Lamb

and Richards (1971b) examined the effect of temperature and desiccation on basidiospores of *Suillus granulatus, Rhizopogon roseolus,* and *Pisolithus tinctorius;* chlamydospores of three unidentified symbionts of *P. elliottii* and *P. radiata;* and oidia of *Xerocomus subtomentosus.* The hyphae of six of these fungi were killed by a 48-hour exposure to temperatures between 28° and 38°C and that of *P. tinctorius* by 45°C. [In other studies Marx *et al.* (1970) found no growth of hyphae of *P. tinctorius* above 40°C.] The chlamydospores were killed by temperatures of 32° to 36°C, the oidia by 47°C, and the basidiospores by 46° to 54°C. Unfortunately, it is not known if the *X. subtomentosus* culture was of the litter-decomposing or of the mycorrhizal type for these can differ substantially in growth properties (Lundeberg, 1970). Chlamydospores did not survive storage at 50% relative humidity for 60 days, whereas basidiospores maintained viability at 30% relative humidity. The resistance of basidiospores (and the susceptibility of mycelia) to relatively high soil temperatures is particularly relevant to seeding in subtropical and tropical climates where summer temperatures of 64.6°C at the surface and 45.3°C at a 5 cm depth have been recorded (Ramdas and Dravid, 1936) and where temperatures in excess of 40°C can frequently occur at 2.5 cm for up to 6 hours (Bowen and Kennedy, 1959, latitude 27°S).

At the other extreme, Moser (1958) recorded variation between different mycorrhizal fungi in resistance of mycelia to temperatures of −11° to −12°C (on agar) and advocated selection of fungi on this basis for alpine reafforestation. Fries (1943) found storing of spores of mycorrhizal fungi at −10°C prolongs their viability, and spores thus provide a survival mechanism in sites even where the mycelium is killed by high or low temperatures.

Resistance of basidiospores to drying has great ecological importance in natural wind-borne dispersion and also has technological advantages in inoculation practices. Theodorou (1971) showed the efficacy of spore inoculation of *P. radiata* seed in the field in both fumigated and nonfumigated soil, and Table I (Theodorou and Bowen, 1973) shows (i) that freeze-dried basidiospores can act as effective inocula 3 months later, (ii) that spores inoculated to seed and dried for 2 days are effective, and (iii) that spores added to soil and dried for 2 months can still lead to good mycorrhizal production.

B. Germination of Propagules

Fries (1966) has reviewed germination of basidiospores. Germination of basidiospores of mycorrhizal fungi has been a rather neglected field of study because if the spores can be made to germinate at all in

TABLE I

EFFECTS OF DRYING ON BASIDIOSPORE INOCULA FOR *Pinus radiata*[a]

Treatment	Total No. short lateral roots	Percent mycorrhiza[b]
A. Uninoculated seed	219	2
Seed inoculated with freeze-dried basidiospores, 3 months old[c]	262	43
B. Uninoculated soil	336	0
Seed inoculated with basidiospores and sown immediately[c]	348	66
Seed inoculated, dried for 2 days[c] before sowing[c]	404	64
C. Uninoculated soil	146	0
Basidiospores in dry soil for 2 months at 25°C[d]	231	41

[a] In all the experiments four replicate pots each with four plants were grown for 4 months.

[b] Percentage of short roots becoming mycorrhizal.

[c] Basidiospores of *R. luteolus* were applied to seed at approximately 5×10^6 spores/seed. Experiment A was in nonsterile soil and B and C were in sterile soil.

[d] 3×10^4 spores/pot.

laboratory media, only a very low percentage does so (often less than 0.1%) and germination is very slow. The percentage of germination of basidiospores can vary considerably from one fruiting body to another. Most studies with mycorrhizal fungi have been restricted to either attempting spore germination of hymenomycetes and gasteromycetes in simple solutions, or on synthetic media with growth substances added, although Melin (1959) added excised roots to synthetic media. In the most detailed study of germination of basidiospores of mycorrhizal fungi yet made, Fries (1943) found even within the one genus basidiospores of some species germinated in distilled water only, some needed the addition of malt extract, others were considerably assisted by diffusates from the yeast *Torulopsis sanquinea*, and others could not be germinated at all. As a rule, *Suillus* spores could be germinated by adding unidentified activators from yeast. However, Fries (1966) subsequently reported that *S. luteus* spores would germinate in a simple glucose–mineral salts solution with ammonium tartrate on thoroughly washed agar, but that activators reduced the germination time from 3–4 weeks to 1 week. Fries found no need for a period of "maturation" of the spore before it would germinate. It is obvious from his studies that inorganic composition of laboratory media affects spore germination profoundly.

Lamb and Richards (1971b) have also found an unidentified activator

(from *Rhodotorula glutinis*) for germination of basidiospores of *S. granulatus*, *R. roseolus*, and *P. tinctorius*. Germination of oidia of their *Xerocomus subtomentosus* culture did not require an activator. Oidia of this fungus could be produced in culture in large numbers, and as they have a broad temperature and pH range for germination and are very resistant to desiccation, if such strains are also mycorrhizal they have considerable attractiveness for inoculum production. Germination of basidiospores of *S. granulatus*, *R. roseolus*, and *P. tinctorius* was optimal at pH 5–5.5. With the first two fungi germination was restricted mainly to pH 4–6.5 but with *P. tinctorius* germination had a somewhat wider range. Although all three fungi had optimum germination at 20°–25°C, germination of *P. tinctorius* occurred between 5° and 40°C, a considerably wider range than the other two fungi, whose germination range was 10°–30°C. Because the chemical composition of the rhizosphere is likely to vary with temperature (Theodorou and Bowen, 1971a) it is difficult to extrapolate from laboratory temperature studies to germination around roots in soil; Lamb and Richard's finding that six times as many spores of *R. roseolus* and *S. granulatus* germinated at 20°C than at 10°C suggests a need for heavier inoculation with spores in many cool-temperature soils in inoculation programs.

A number of organisms can produce stimulators to germination and these include plant roots, yeasts, various soil fungi, and pieces of fruiting bodies. Fries (1943) found that the mycorrhizal fungus *Cenococcum graniforme* could stimulate spore germination of three *Suillus* species in laboratory culture. However, as metabolites produced in laboratory media are almost certainly quite different from those produced in the soil, the ecological importance of such a finding is doubtful. The stimulatory effects of growing roots on basidiospore germination has obvious ecological implications. Basidiospores of *Russula* have proven very intractable to germination on usual laboratory media, but Melin (1959) was able to induce germination of some (but not all) species by addition of excised pine roots to a nutrient medium containing B vitamins and amino acids. Sometimes tomato roots were also effective but in other instances pine roots were needed, possibly suggesting at least quantitative differences if not qualitative differences in stimulator requirements of spores of different fungi. Melin also showed excised roots to stimulate spore germination of species of *Suillus*, *Amanita*, *Paxillus*, *Cortinarius*, and *Lactarius*. Failure to obtain germination by adding excised roots to synthetic media may reflect a toxin production by the roots, a toxin in the medium, or a need for factors from living roots; Marx and Ross (1970) were unable to induce basidiospore germination of *Thelephora terrestris* with root extracts or detached roots of *P. taeda* but

the spores germinated to form mycorrhizae with intact roots of *P. taeda* in axenic culture.

The nature of the activator for basidiospore germination is still unknown; several compounds may be involved. The activator yeast is usually separated from the basidiospore on an agar plate, and Fries (1966) suggested therefore, activators must be readily diffusible. The removal of the stimulus by removal of the activator organism suggests involvement of volatile compounds, a very labile compound, or one readily used by spores. Modern analytical tools such as gas chromatography have not yet been employed in these studies.

Fries (1966) pointed out germination stimulation can also be due to removal of toxins in agar media and that asparagine prevents germination of *S. luteus* spores. It is of some interest therefore that asparagine has been recorded in exudates from tree roots (see Table II).

Placing these studies on basidiospore germination in a wider context of rhizosphere biology, plant root stimulation of germination of spores and sclerotia of plant pathogenic fungi is well known (Warcup, 1967). In some such cases specific substances may be involved but in others the response is of a more general nature, for the rhizosphere environment differs from soil in factors such as pH, chemical composition, and oxygen and carbon dioxide concentrations as well as being a zone of intense microbial activity (see Section IV). Some stimulators could be due to the rhizosphere microflora rather than the plant root.

Another soil phenomenon difficult to accommodate in laboratory studies of germination is mycostasis (Dobbs and Hinson, 1953). Such fungistasis is thought to be due to metabolites from other soil organisms but chemical identification of these metabolites has seldom been made (see Garrett, 1970; Lockwood, 1964). Fungistasis can be overcome by addition of readily available energy sources, e.g., glucose, which occur in most root exudates (see Table II), and the triggering of germination by the proximity of a root probably has great conservation value for fungi such as mycorrhizal fungi which seem to be largely restricted to growing on roots. Where specificity of roots in stimulating spore germination occurs (Melin, 1959) greater conservation advantages would occur. Germination of basidiospores of mycorrhizal fungi around plant roots *in soil* is an area much in need of further study.

III. Growth around Seeds

Germinating seeds liberate sugars, amino acids, and other substances into the spermatosphere and these lead to increased microbial activity

around the seed (Picci, 1959; Fries and Forsman, 1951). However not all seed diffusates are stimulatory, and Bowen (1961) showed that seed diffusate of the legumes *Centrosema pubescens* and *Trifolium subterraneum* contain an antibiotic toward a wide range of gram-negative and gram-positive soil bacteria. Some organisms were insensitive to the toxin and were stimulated by the seed diffusate. Ferenczy (1956) recorded seed diffusates toxic to gram-positive bacteria from species of *Abies, Picea, Pinus,* and *Pseudotsuga.*

Melin (1925) observed growth stimulatory substances for mycorrhizal fungi coming from germinating pine seeds. Similarly, we have observed that mycelia of *R. luteolus* and S. *granulatus* will grow well on the seed coat of *P. radiata* seeds germinating on water–agar. The extremely limited study of growth of mycorrhizal fungi around seeds would suggest seed diffusates to be stimulatory and not inhibitory to them. It is not known what happens to growth of mycorrhizal fungi around seed in soil with other microorganisms present. If considerable growth of inoculated fungi took place around the seed this would lead to a higher inoculum potential for early colonization of the emerging radicle.

Much spore or mycelial inoculum adhering to the testa is often wasted when the testa is carried above the soil, following germination. The success of spore inoculation of pine seed (Theodorou, 1971) indicates that sufficient spores remain around the emerging root to establish infection, but in seed inoculation some advantage will lie in incorporating inoculum in materials which detach from the testa soon after planting.

IV. Growth around Roots

It is necessary to sustain growth of the fungus in the rhizosphere for at least some weeks after sowing before infection occurs. In nursery soil in Finland, with mean air temperatures around 15°C, Laiho and Mikola (1964) first observed mycorrhizal infection of pine and spruce 6 to 7 weeks after sowing; under probably colder soil conditions in England, Robertson (1954) first observed mycorrhizae on *P. sylvestris* some 18 weeks after sowing in March. The reasons for delay in infection are unknown. The suggestion that sufficient carbohydrate is not available for the fungus until photosynthesis rates become high with the advent of the first true leaves, by itself does not seem tenable, since the fungus grows in the rhizosphere on root exudates well before this.

Marked ectotrophic growth (according to Garrett, 1970) around and along roots precedes infection of pine, spruce (Laiho and Mikola, 1964), beech (J. Warren-Wilson, in Harley, 1969), and *Eucalyptus* (Chilvers

and Pryor, 1965). Chilvers and Pryor also considered spread of infection along and between roots of *Eucalyptus* was by surface growth, whereas both internal and external growth are important in spread of the infection with pine and spruce roots (Laiho and Mikola, 1964; Robertson, 1954; Wilcox, 1968). With root-infecting pathogens, ectotrophic growth usually precedes infection of underlying cells, and the ectotrophic growth habit reduces host resistance to infection. A full discussion of ectotrophic growth of pathogens is given by Garrett (1970), who considered a concept of ectotrophic growth overcoming host resistance inappropriate to the harmonious relationships exhibited by an ectomycorrhizal fungus and its host. However, to discard such a concept for ectomycorrhizae is premature in the absence of any detailed study on infection and developmental processes of ectomycorrhizae. It is interesting that in the spectrum of microorganism–plant interactions from noninfecting rhizosphere microorganisms to mycorrhizae to plant pathogens, there are marked similarities in many properties which can often be distinguished (Bowen and Rovira, 1969).

Our interest in ectotrophic growth on roots arose from studies on establishing inocula around roots. However it soon became apparent that most, or all, previous studies on the effects of environment on mycorrhizal production did not distinguish between the two fundamental steps: (i) effects on fungus growth around seedling roots prior to infection and (ii) effects on infection processes and subsequent mycorrhizal development (see Chapter 1). It also became apparent that there was a great lack of experimental approach toward quantitatively evaluating existing concepts and enunciating new ones in the "microecology" of fungi on roots.

This section deals first with a definition of the rhizosphere environment for growth, then with experimental approaches to fungus colonization of roots, and lastly with the dynamics of microbial colonization of roots in soil.

The root in soil is a dynamic, interacting, three-compartment system in series, i.e., the soil, a somewhat discontinuous microbial layer, and the plant root itself. The "rhizosphere" was defined by Hiltner (1904) as the region of soil around a root in which a root influence on microflora occurred. However the extent of this influence will depend on the amount of substances lost from roots, on the rate of diffusion of soluble and volatile materials away from the root in the particular soil, and on the sensitivity of different organisms to diffusates from the root. The term "rhizoplane" is applied to the immediate soil–plant interface, and "rhizosphere" is applied to an ill-defined narrow zone of soil (about 1–3 mm wide) surrounding the root and root hairs. Here we will refer

to these collectively as the rhizosphere. It differs substantially from the soil around it because (i) the intact root loses organic substances—"root exudates"—thus stimulating microbial activity greatly. (In soil microbiology literature the term "root exudates" is distinct from other plant physiological connotations which refer to bleeding sap of decapitated roots.) (ii) The roots secrete inorganic ions, including hydrogen ions and bicarbonate ions. (iii) Carbon dioxide and oxygen concentrations change as a result of root and microbial respiration. (iv) Solutes in the soil move to the roots in water or by diffusion; some may be completely depleted because of high uptake by the root and others may accumulate. (v) The root frequently sloughs old material such as root cap and epidermal cells which release their contents (see Chapter 8).

Certain groups of soil organisms are selectively stimulated in the rhizosphere (see Rovira, 1965), in part owing to the above factors. Similarly the immediate environment of fungal hyphae and mycorrhizae is rather different from surrounding soil and adjacent uninfected roots, thus leading to increased microbial activity and microbial specificity in the "mycorrhizosphere" (Katznelson *et al.*, 1962; Neal *et al.*, 1964; Foster and Marks, 1967; Oswald and Ferchau, 1968).

A. Root Exudates of Trees

A number of organic compounds capable of being used as energy and growth factor sources for microorganisms have been recorded to come from living plant roots, e.g., sugars, amino acids, vitamins, organic acids, nucleotides, flavonones, enzymes, terpenes, and many unidentified compounds (see Chapter 8).

1. Methods of Study and Sites of Exudation

Most root exudate studies with tree species have involved collection of exudates over several days, using nonradioactive or radioactive techniques. The plants are grown under sterile conditions, usually in solution or sand culture, from which the exudates are collected at intervals of several days, concentrated, and analyzed. In contrast to collections over very short periods (possible with radioactive tracers) this exudate is "net loss" from the root, i.e., the balance between actual loss and reabsorption by the plant, since it is well known that most solutes move both in and out of cells, i.e., influx and efflux, respectively (Briggs *et al.*, 1961). Such exudate collection will not detect labile compounds or volatile compounds not trapped by the growth medium and will often include material lost from senescent and dying cells. The use of radio-

isotope labeling such as $^{14}CO_2$ used by Subba-Rao *et al.* (1962) with tomato and Slankis *et al.* (1964) with *P. strobus* increases the sensitivity of detection of exudates and sites of exudation considerably. Using 9-month-old *P. strobus* seedlings, Slankis *et al.* (1964) found some 0.8% of $^{14}CO_2$ supplied was recovered in the solution bathing the root during 8 days, and considering the very large effects of growth conditions on exudation, this is of the same order as found with young herbaceous plants (Rovira, 1969a).

With annual plants, seedlings are usually grown with their roots continuously in sterile media (Rovira, 1965). Collection of exudates from mature root systems of trees poses a special problem; W. H. Smith (1970) obtained exudates from mature sugar maple by air layering, then surface sterilizing new roots and aseptically introducing them into sealed sterile test tubes containing nutrient solution. As Smith pointed out, the possibilities of root damage by the sterilizing agents must be minimized, and he surface sterilized roots with a 10–15-second submersion in an antibiotic solution of cycloheximide, streptomycin sulfate, and griseofulvin. Changes in permeability of roots due to sterilizing agents should be easily checked by preliminary experiments using loss of ^{36}Cl as an indicator, as efflux of this is usually passive.

"Pulse" labeling with $^{14}CO_2$ has been employed by MacDougall and Rovira (1965, 1970), Rovira (1969b), and MacDougall (1970) with wheat over much shorter times than those above. Collection of exudates over very short periods, e.g., a few minutes to a few hours, measures exudation relatively free of reabsorption and of loss by senescing cells but will record exudation only from metabolic pools in the root which have become labeled with ^{14}C. Unless all such pools are labeled to the same specific activity, comparison of amounts of different compounds exuded could be in error. A solution to this problem is to grow plants in an atmosphere including $^{14}CO_2$ continuously to ensure uniform labeling as much as possible, or to allow a long period between labeling and collection of exudates.

Exudates are usually collected from solution-grown plants or as eluates of sand culture systems, but occasionally synthetic soil systems have been used. Considering the markedly different physical appearance of roots grown in solution and grown in sand it is not surprising (in retrospect) that Boulter *et al.* (1966) found large differences in exudation from pea roots grown in culture solution and sand culture; up to 700% increase of some amino acids was found with plants grown in quartz sand. Some workers have examined leachates of planted nonsterile soils (e.g., Harmsen and Jager, 1963; Martin, 1971) but such leachates may be partly of microbial origin and partly of root exudates

TABLE II Root Exudates of Tree Species and Wheat

	Pinus strobus[a] 1	*Pinus lambertiana*[b] 2	*Pinus banksiana*[b] 3	*Pinus rigida*[b] 4	*Pinus radiata*[b] 5	*Pinus radiata*[c] 6	*Pinus resinosa*[d] 7	*Robinia pseudoacacia*[b] 8	*Acer saccharum*[e] 9	*Eucalyptus pilularis*[f] 10	Wheat[g]
Amides and amino acids											
α-Alanine		−[h]	−	+[i]	+	+	+	−	+	+	+
β-Alanine											+
γ-Aminobutyric acid		+	+	+	+	+	+	−		+	+
Arginine						+					
Asparagine	+	−	−	−	−	+	+	+			+
Aspartic acid	−	+	+	+	+	+	+	+		+	+
Cystathionine											+
Glycine		+	+	+	+	+	+	+	+	+	+
Glutamic acid	−					+	+		+	+	+
Glutamine	+					+	+		+	+	+
Homoserine									+		
Leucine/isoleucine		−	+	+	+	+	+	+	+		+
Lysine							+				+
Methionine		−	−	−	−			+	+		+
Proline						(+)[j]	+				+
Phenylalanine		+	+	+	+	(+)		−			+
Serine		+	+	+	−	+	+	+	+		+
Threonine		+	−	+	−	+	+	−	+		+
Tyrosine		−	−	−	−			+	+		+
Valine						+	+		+	+	+
Typtophan						+					
Sugars											
Arabinose	+						+			+	+
Fructose		+	−	−	−		+	−	+	+	+
Galactose										+	+
Glucose	+	+	+	+	+		+	+	+	+	+

Oligoscaccharides										+
Raffinose										+
Rhamnose		+	−	+	−		+	+	+	+
Ribose								+		+
Sucrose		+	−	+	−		+	+		
Xylose									+	
Organic acids										
Acetic		+	+	+	+		+	+		+
Butyric										+
Citric								+	+	+
cis-Aconitic	+									
Fumaric		−	+	−	−		−			+
Glycolic	+				+					+
Malic	+								+	+
Malonic	+	+	−	−	−		+	+		
Oxalic	+	+	+	+	+					+
Oxalacetic			+	−			−	+		
Propionic										+
Shikimic	+									
Succinic		+	+	+	+		+		+	+
Valeric										+

[a] Slankis *et al.* (1964).
[b] W. H. Smith (1969).
[c] Bowen (1969).
[d] Agnihotri and Vaartaja (1967).
[e] W. H. Smith (1970).
[f] Cartwright (1967).
[g] Rovira and MacDougall (1967).
[h] Minus sign designates absence.
[i] Plus sign designates presence.
[j] (+) designates present in some unreported studies.

not decomposed or absorbed by the soil and its associated microorganisms.

Pearson and Parkinson (1961) with nonradioactive experiments, MacDougall (1968) with ^{14}C, Bowen (1968) with ^{36}Cl, and Rovira and Bowen (1970) with ^{32}P allowed exudation onto moist filter paper on either side of a root and thus located sites of loss from the root. In the last three cases, counting of radioactivity along the root and the filter paper enabled loss to be calculated as a fraction of radioactivity in each part of the root. Subsequent one-dimensional chromatography of the filter paper strip enables chemical characterization of the radioactive exudates. Greatest exudation usually occurs from the elongating zone of the root from a few mm to 2–5 cm from the root apex, but appreciable loss can occur all along the root, especially where root damage occurs, such as from emergence of lateral roots, microfauna feeding, and mechanical damage (see Rovira, 1969a). In an extension of the filter paper method, Rovira (1969b) showed peaks of loss of ^{14}C labeled substances near the apex of wheat roots were largely due to poorly diffusible substances which may be the mucigel described by Jenny and Grossenbacher (1963). Readily diffusible exudates occurred in approximately equal amounts along the whole of 20-cm root studied. By sowing spores of nutritional mutants of the fungus *Neurospora crassa* along sunflower roots, Frenzel (1960) showed some amino acids were lost from the root tip and others from the root hair zone.

2. *Composition of Tree Root Exudates*

Table II summarizes the present records of exudates from forest tree species; wheat data has been included for comparison. As for other plants a wide range of sugars, amino acids, and organic acids have been detected in root exudate from tree species. Although the lists must be considered far from complete—the main compounds investigated are those for which analytical techniques are well developed—they do include the most abundant groups of soluble chemical components of plant cells. In addition, W. H. Smith (1969) reported the vitamin, niacin, in *P. radiata* exudates.

The neglect of volatile compounds in root exudate studies may have seriously underestimated the extent of exudation. Rovira and Davey (1972) referred to wheat studies in which for every unit of carbon exuded as water-soluble material, some 3–5 units were released as non-water-soluble mucilaginous material and root cap cells, and some 8–10 units of materials volatile under acidic conditions were released; much of the volatile material was probably carbon dioxide. Krupa and Fries (1971) and Melin and Krupa (1971) have recently demonstrated the main volatiles of root extracts of *P. sylvestris* to be mono- and sesquiter-

penes but no examination of root exudates appears to have been made; substances such as terpenes may well affect the microbial composition of the rhizosphere.

Within the one set of conditions (e.g., W. H. Smith, 1969) exudates may vary both qualitatively and quantitatively between species (Table II, columns 2–6). Smith also showed the amount of exudate in seedlings was related to size (see Table III) but this only partially explains the species differences. Richter *et al.* (1968) and Rovira (1969a) have reported extremely large variation in amounts of exudates (up to a hundred- or thousandfold differences) released by a species depending on the plant growth conditions and the method of collection of exudates. Within the one species under different conditions large differences in exudate composition occur (Table II, columns 5 and 6).

For exudates from forest tree species the only detailed quantitative comparison between amino acids, sugars, and organic acids was made by W. H. Smith (1969, 1970) who showed organic acids were by far the major component of the exudates of both seedlings and mature trees (Tables III and IV). Slankis *et al.* (1964) found the major exudate in their studies was malonic acid. The concentration of particular exudates in the rhizosphere in soil will depend on their rate of movement from the root by diffusion and their uptake by the rhizosphere microflora.

3. *Mechanisms of Exudation*

Mechanisms of exudation of organic compounds have not been studied in detail but generally loss is assumed to occur by passive leakage. Studies of loss mechanisms should be placed in a strict biophysical framework (see Chapter 5) viz., passive loss is loss down a concentration gradient (for uncharged solutes) or an electrochemical potential gradient

TABLE III

EFFECT OF SPECIES ON EXUDATION[a]

Species	Plant dry wt. (mg)	Root dry wt. (mg)	Carbohydrates (μg/seedling)	Amino acids (μg/seedling)	Organic acids (μg/seedling)
P. radiata	21.1	3.6	13.0	149	320.7
P. lambertiana	64.1	9.6	33.8	301	546
P. banksiana	6.4	1.9	0.1	64	270
P. rigida	8.0	1.9	1.0	29	66
Robinia pseudoacacia	14.8	4.2	9.0	44.3	77

[a] Data of W. H. Smith (1969). Exudation over 10 days in complete nutrient solution.

(for charged ions), the rate of loss being a function not only of the gradient but also the permeability of cell membranes to the particular solute. "Active" processes move against the electrochemical potential gradient. For most organic exudates there is almost certainly a lower electrochemical potential on the outside of the root than inside the cells, thus suggesting a purely passive leakage of exudates, but permeability needs to be considered also with regard to *rates* of loss. Cirillo (1961) has described carrier-facilitated diffusion for entry of sugars into yeast cells down a concentration gradient but this possibility does not appear to have been explored with loss of organic substances by higher plants, and it seems unlikely.

Results of Boulter *et al.* (1966) with pea indicate that analyses of exudates do not always precisely reflect the composition of the root, for much depends on where solutes are localized in the cell and on the modes of loss of different solutes and the membrane permeability to each solute. Nevertheless, some relationship between root exudates and cell composition would be expected, and in future more cognizance could well be given to the effects of various treatments on cell composition when studying root exudate composition, e.g., high potassium or high cation media are well known to lead to accumulation of organic acids in plant cells, and although most of this is in the vacuole, we may well expect an increase in organic acids in the root exudates as well. Following Krupa and Fries' (1971) studies on *P. sylvestris* exudates we might reasonably expect some mono- and sesquiterpenes in its root exudate but little ethanol, acetoin, or isobutyric acid.

4. Conditions Affecting Exudation

Rovira (1969a) has indicated factors affecting root exudation by a range of plants. Below we apply these to studies on tree species.

a. Plant Age. W. H. Smith's results (1970) (Table IV) show that although seedling sugar maple (*Acer saccharum*) produced a wider range of sugars and more sugar than did young roots on a 55-year-old tree, sugars and amino acids were minor components of the exudate compared with organic acids, especially in the mature trees. Roots of these produced six times more organic acids/mg dry weight than did seedling roots. Acetic acid was the major organic acid in both instances; in addition, roots on mature trees exuded citric and malonic acids and seedling roots lost oxalacetic acid. Bowen (1964) found the amino acids in root exudates of *P. radiata* seedlings decreased from 2 to 6 weeks but the amino acid composition of the exudates did not change.

b. Nutrition. Nutrition effects on root exudation have not been

TABLE IV

EFFECT OF TREE AGE ON EXUDATION BY *Acer saccharum*[a]

Carbohydrates[b]	Seedling	Mature	Amino acids/ amides[b]	Seedling	Mature	Organic acids[b]	Seedling	Mature
Fructose	9.8 ± 1.5[c]		Alanine	0.2 ± 0.1	2.4 ± 0.2	Acetic	67.3 ± 5.0	495.7 ± 18.2
Glucose	4.7 ± 0.8	0.5 ± 0.1	Glutamine	0.3 ± 0.1	0.3 ± 0.1	Citric		46.7 ± 4.5
Rhamnose	0.7 ± 0.1		Glutamic acid		0.2 ± 0.1	Malonic		12.0 ± 2.5
Ribose	1.2 ± 0.1		Glycine	0.2 ± 0.1	0.8 ± 0.1	Oxalacetic	23.3 ± 2.7	
Sucrose	20.6 ± 2.1	5.8 ± 1.4	Homoserine		0.5 ± 0.1			
			Leucine/isoleucine	0.4 ± 0.1	1.4 ± 0.2			
			Methionine	0.2 ± 0.1	0.6 ± 0.1			
			Phenylalanine	0.7 ± 0.1	1.6 ± 0.1			
			Serine	0.2 ± 0.1	1.3 ± 0.2			
			Threonine	0.8 ± 0.1	1.7 ± 0.1			
			Tyrosine		0.8 ± 0.1			
			Valine	0.2 ± 0.1	0.6 ± 0.1			

[a] From W. H. Smith (1970), by permission of *Phytopathology*.

[b] Data in mg × 10^{-4} of each carbohydrate, amino acid/amide, and organic acid released per mg of oven dry roots of maple seedlings or mature tree during a 14-day growth period.

[c] Mean and standard error of three replicate determinations using one composite exudate sample from 200 seedling or 20 mature tree roots.

extensively studied. Bowen (1969) found phosphate deficiency in *P. radiata* led to two and a half times more amino acid/amide in exudate than from control plants, and nitrogen-deficient plants exuded only a quarter as much amino acid/amide as control plants, independent of effects on growth (Table V). Cartwright (1967) found that varying the nitrogen and phosphorus levels in culture solutions had no effect on organic acid exudation of *Eucalyptus pilularis* seedlings. Amino acid changes were consistent with those above while the quantity of sugars exuded increased both with decreasing phosphorus supply and decreasing nitrogen supply. Maltose and fructose were detected only at high nitrogen levels (70 and 140 mg N/liter) and galactose only at a low nitrogen level (14 mg N/liter). Nitrogen was supplied as nitrate.

The increase in net amino–amide N loss from *P. radiata* with low phosphorus was examined further by Bowen. Phosphate level had no effect on uptake of ^{14}C labeled amino acids, thus eliminating the possibility that the greater exudation of phosphorus-deficient plants was due

TABLE V

EFFECT OF NUTRITION ON AMINO ACID–AMIDE EXUDATION OF *P. radiata* SEEDLINGS[a]

Amide or amino acid	Nutrient solution		
	Complete	Phosphate deficient	Nitrogen deficient
Asparagine	10.9[b]	32.5[b]	3.0[b]
Glutamine	23.6[c]	52.0	2.8
γ-Aminobutyric acid	5.2	13.8	1.0
α-Alanine	1.6	2.8	1.2
Aspartic acid	4.4	9.6	2.0
Glutamic acid	6.0	19.7	2.0
Glycine	7.3	14.0	3.4
Leucine	3.0	5.6	1.8
Serine	4.8	8.0	2.0
Threonine	1.4	2.0	—
Valine	1.8	4.0	0.1
Total amido/ amino nitrogen[d]	104.5	248.5	25.1

[a] From Bowen (1969), by permission of *Plant and Soil*. Amide and amino acid exudates from *Pinus radiata* seedlings 2–4 weeks. Roots were of similar length in all treatments.

[b] Moles $\times 10^{-9}$/plant.

[c] Some arginine was also present but only in small amounts.

[d] Including the two NH_2 groups of asparagine and glutamine.

to a greater reabsorption of lost amino acids by phosphorus-sufficient plants. An effect of phosphate deficiency on increasing permeability of root cells was eliminated by examining the passive loss of ^{36}Cl from pine roots, which was the same for both phosphorus-sufficient and -deficient plants. However phosphorus deficiency gave a doubling of free amino–amide nitrogen in the root and this was the most likely reason for the increased exudation of amino acids and amides with low phosphorus plants.

c. Light. With herbaceous plants, shading has decreased amino acid exudation, but sugars and organic acids have apparently not been studied. The only tree species information is indirect, e.g., Harley and Waid's finding (1955) of different proportions of *Trichoderma* and *Rhizoctonia* on roots of beech, depending on the light regime.

d. Temperature. As with light, effects of temperature on root exudation by *P. radiata* can be deduced by an inordinate reduction of colonization by some strains of *R. luteolus* when reducing soil temperatures from 20° to 15°C compared with growth in laboratory media (Theodorou and Bowen, 1971a, see Section G,4, Table VII).

e. Microbial Effects. Microbial effects on exudation are the most difficult to study. This question is of crucial importance because if microorganisms greatly affect exudation, most of the studies carried out under asepsis, have to be qualified. Furthermore, effects by specific organisms will greatly enhance the competitive ability of that organism in the rhizosphere. Rovira (1969a) derived from Harmsen and Jager's results (1963) that exudation from wheat roots into synthetic soil was increased at least fourfold by microorganisms.

Possible ways in which microorganisms could affect root exudates are (i) by producing substances affecting root permeability. Various antibiotics produced by soil microorganisms in culture can increase permeability (Norman, 1955, 1961), but whether these compounds are produced in sufficient quantity in the rhizosphere to have such effects is yet unknown. (ii) By affecting root metabolism. This may affect the concentration of solutes in the cell or may affect loss mechanisms directly. Metabolic effects of rhizosphere microorganisms have been indicated by Bowen and Rovira (1969) and the phenomenon is well known with plant pathogen-host interactions. Mycorrhizal fungi are known to produce hormones and cytokinins; Highinbotham (1968) found hormones to increase permeability of *Avena* coleoptiles and an increase in passive efflux of potassium. D. Smith *et al.* (1969) noted that the translocation stream of autotrophic higher plants is diverted toward

the site of association with fungi, including mycorrhizae. Krupa and Fries (1971) recorded a two- to eightfold increase in volatile compounds in roots of *P. sylvestris* which were mycorrhizal. (iii) Where loss of a solute is by passive diffusion, thought to be the usual case, absorption of exudates by microorganisms would maintain a steep concentration gradient (or electrochemical potential gradient) to drive solute diffusion. The movement of carbohydrates from beech roots to the mycorrhizal sheath, studied so well by D. H. Lewis and Harley (1965a,b,c) is a special and striking case of microorganisms external to cells (i.e., the Hartig net and the fungus mantle) affecting loss of solutes from cells and is directly analogous to a rhizosphere microorganism situation. The mechanism of the loss, as with other symbioses, is unknown but removal of sucrose by the fungus and elaboration into trehalose and polyols maintains a steep concentration gradient for further sucrose loss. D. Smith *et al.* (1969) have indicated a specificity in transfers of carbohydrates in symbiotic systems.

B. Organic Compounds Other Than Root Exudates

Very little information exists on organic substances in the rhizosphere other than root exudates. Reference has been made above to loss of organic substances by senescence of root cap cells or sloughed epidermal cells. Rovira (1956a) found pea roots released 0.5 mg dry weight of cell debris per plant over 21 days. No data are available for forest tree species, although sloughing of epidermal cells from seedling pine roots is very marked.

Small amounts of free organic acids and amino acids probably occur in soil, although analytical techniques usually do not distinguish between such substances in free form and those occurring from death of microorganisms during extraction procedures. Moodie (1965) has suggested that movement of such substances in soil toward roots by convection in water might be important to the nutrition of rhizosphere microorganisms, but the concentrations of free amino acids and organic acids in soil are probably too small for this to be significant. Products of microorganisms growing in the rhizosphere will also contribute to the rhizosphere's chemical composition.

C. Inorganic Composition of the Rhizosphere

Inorganic compounds in the rhizosphere do not usually serve as an energy source and therefore have not attracted study by microbiologists. Measurements of oxygen and carbon dioxide concentrations in the rhizosphere have received little attention, although there is general agreement that oxygen levels will be lowered and carbon dioxide levels raised by

such processes as respiration by roots and microorganisms. The metabolic activities of the root itself will not be impaired until the oxygen concentration at the surface of the root is very low (Greenwood, 1969). The isolation of *Clostridium* from roots indicates that small anaerobic pockets do occur on root surfaces.

Soil chemists interested in ion movement to roots have studied the inorganic composition of the rhizosphere. Ions move to roots by convection in water (mass flow) and diffusion, and depending on the absorbing power of the root for that particular ion, it will be completely absorbed or will accumulate around the root. Thus phosphate concentrations in the rhizosphere are usually much lower than in soil solution (D. G. Lewis and Quirk, 1967), while Barber and various associates (Barber, 1962; Barber *et al.*, 1963; Barber and Ozanne, 1970) showed autoradiographically that ^{90}Sr, ^{35}S, and ^{45}Ca usually accumulate around roots. In the studies by Barber and Ozanne (1970), *Lupinus digitatus* absorbed enough calcium to cause depletion around roots. Riley and Barber (1969, 1970) showed an approximate doubling of soluble salts in the rhizosphere soil around soybean (1–4 mm distant from the root) and approximately sixfold increases at the rhizoplane. The rhizoplane soil was 0.3–1.3 pH units higher than surrounding soil. This pH increase was probably due to bicarbonate efflux from the roots during nitrate absorption. Where ammonium ions supplant nitrate, and also when high cation absorption occurs, hydrogen ions tend to be lost from the root thus decreasing pH, and in sand cultures a pH of 3 has been recorded with ammonium nutrition (Nightingale, 1934).

Many inorganic ions as well as bicarbonate, hydroxyl, and hydrogen ions are lost by roots. Using the filter paper method above, Bowen (1968) demonstrated loss of 7.2% of the chloride from the apical 2–3 cm of *P. radiata* seedling roots and 3.3% from basal parts of the roots. Tracer studies of this type measure efflux and need not be interpreted as leading to an accumulation of these ions in the rhizosphere; where influx exceeds efflux there is no net accumulation of the ion in the rhizosphere.

D. Growth on Surfaces

The microenvironment immediately around surfaces can be markedly different from bulk solution (e.g., Mitchell, 1951). Dynamics of growth along and on surfaces have been little studied. Most classical microbiological studies of growth kinetics have been made in stirred solutions.

E. Root Exudates and Growth of Mycorrhizal Fungi

Any treatment of growth of mycorrhizal fungi around roots would be quite incomplete without reference to Melin's extensive studies on root

substances and nutrition of mycorrhizal fungi (see Section II,B also). Melin (1963) reviewed his studies on growth of mycorrhizal fungi in culture which showed (i) growth of many mycorrhizal fungi in culture was considerably enhanced by addition of vitamins and amino acids. (ii) Growth of many fungi was enhanced still further by incorporation of unidentified diffusates from cultures of excised roots of pine, sterile exudates from attached roots, and extracts from dead roots obtained at 100°C. The term "M factor" has been given to these unidentified compounds. (iii) Exudates of other than pine species, e.g., tomato could stimulate growth of the fungi, and (iv) high concentrations of M-factor preparations were inhibitory to growth. Melin interpreted these results to suggest the presence of both diffusible and indiffusible stimulatory factors and of an inhibitory factor. It is possible that the stimulation of growth he observed immediately around killed extracted roots was an inert surface effect, i.e., a thigmotropic response which commonly occurs with fungi and other microorganisms. However studies by Melin and others on root colonization of live pine seedlings (e.g., Theodorou and Bowen, 1969) clearly indicate the stimulation of growth of mycorrhizal fungi by root exudates, free of considerations of the thigmotropic response. Melin's studies and other studies of nutrition of mycorrhizal fungi (see Harley, 1969) were made on laboratory media and the search for the identity of the M factor(s) has been pursued without success using replacement techniques.

What do root exudate analyses have to contribute to fungal growth studies in synthetic media?

(i) Amino acids and sugars readily used by mycorrhizal fungi (Harley, 1969; Palmer and Hacskaylo, 1970; Lundeberg, 1970) occur in root exudates of tree seedlings and other plants. [This latter point is consistent with Theodorou and Bowen's findings (1971b) that mycorrhizal fungi can grow in the rhizosphere of nonhost plants, e.g., grasses.] W. H. Smith (1969, 1970) has shown an abundance of organic acids in exudates from tree species, but use of organic acids by ectomycorrhizal fungi has received scant attention; Palmer and Hacskaylo (1970) found some ectomycorrhizal fungi can use citrate as a sole carbon source, but acetic acid (as acetate), the most abundant organic acid in Smith's studies, was little used as a pure carbon source by the six fungal isolates they studied.

(ii) So far no identity of the M factor has been advanced. As considerable variation occurs between mycorrhizal fungi in specific growth requirements it would not be at all surprising to find the M factor (if it exists) is not one compound but several. Root exudate analyses reveal examination of such groups of compounds as amino acids and amides as possible M factors have been by no means exhaustive. For

example, media employing casein hydrolysate as the sole amino acid sources include alanine, arginine, aspartic acid, glycine, glutamic acid, leucine, proline, phenylalanine, serine, threonine, valine, and tryptophan, but notable omissions are asparagine, glutamine, and γ-amino butyric acid, all of which are major components in tree seedling exudates (W. H. Smith, 1969; Bowen, 1969). Furthermore, ratios of the various amino acids common to both root exudates and casein hydrolysates are different. Antagonisms between amino acids are also well known (but little understood). Similarly, amino acids in high concentration can be toxic to mycorrhizal fungi: Melin (1963) recorded *Boletus versipellis* and *Lactarius rufus* to be sensitive to all but very low levels of glutamic acid, and Lundeberg (1970) has shown mycorrhizal fungi to be very responsive to asparagine at low levels but toxic at higher levels. Theodorou and Bowen (1968) have found growth of *R. luteolus* to be markedly depressed by 1.15 gm/liter of casein hydrolysate, but that this is relieved by addition of amino acids which are present in exudate of *P. radiata* but absent from casein hydrolysate. In view of these complex interactions and the exhibition of stimulatory and inhibitory effects by the one compound, it is possible that the idea of a specific M factor(s) is illusory; the balance of a number of substances might be the important factor. If specific M factors exist, they may not be easy to find by *ad hoc* replacement techniques alone. To base replacement studies on analyses of exudates is desirable but difficult; analytical techniques are quite laborious and unusual compounds are especially likely to be missed. A further difficulty in relating replacement studies to a real situation lies in demonstrating that the concentration of a particular substance active in laboratory media occurs in the natural situation.

(iii) Mycorrhizal research so far has done little to elucidate the morphogenic phenomenon of mantle formation occurring with many ectomycorrhizae. Melin's studies showed abundant mycorrhizal growth around roots—is this due to an indiffusible M factor? Is mantle development a thigmotropic response and if so, what is the reason for this? Is it due to a nonspecific accumulation of nutrients and energy sources or other environmental conditions at the root–soil interface?[1]

F. Experimental Studies on Ectotrophic Growth along Roots

There are now some data on the effect of soil and biological factors on mycorrhizal production but these rarely distinguish between effects

[1] *Note added in proof.* D. J. Read and W. Armstrong (1972), *New Phytol.* **71**, 49, have found internal oxygen supply to silicone rubber "roots" to be important in sheath formation by *Boletus variegatus*.

on growth in the rhizosphere and on infection processes (and subsequent mycorrhizal development). Extensive data also exist on effects of environmental variables on growth of ectomycorrhizal fungi in laboratory media (see Harley, 1969), but the relationship between such observations and colonization of roots in soil is often not clear. There appear to be no published quantitative data on the rate of ectotrophic growth of mycorrhizal fungi along roots in field soil.

1. Methods

In the last 3 years we have approached colonization of roots in an experimental manner. Our usual analysis of the effects of a particular variable is to examine (i) growth of a range of test mycorrhizal fungi in laboratory media with the nutritional or environmental variable imposed; (ii) growth of the fungus, applied as mycelial inoculum, on the surface of pine seedling roots in soil, usually for 4 weeks (but up to 8), i.e., before infection occurs; and (iii) mycorrhizal production in soil after growth of the test plant for 3–5 months under greenhouse conditions.

Soil conditions may affect the growth of the fungus in the rhizosphere either directly or indirectly via the effects on the plant and root exudation. Colonization studies must also recognize the possibility of surface or thigmotropic effects. For these reasons controls in our colonization studies consist of 1-mm-diameter bundles of 13-μm-diameter sterile glass fibers which are inoculated in soil in precisely the same way as the pine seedling root. Sterile seedlings are planted to sterilized soil in 20 × 3-cm test tubes. The inoculum is applied as growing mycelium in a 3-mm-diameter disk of Melin-Norkrans agar medium (Melin, 1959) from which residual nutrients have been removed by washing in sterile water. After the colonization period, the soil is gently removed from the root either by washing or allowing it to drop off in water. The roots are then stained with 1% cotton blue in lactophenol, mounted in water, and the length of root colonized is measured and a rating (0–4) given for intensity of colonization. Most of the variation is between seedlings, rather than between tubes, and a coefficient of variation of about 15% was obtained by using 10 tubes each with two seedlings per tube. Full details were given by Theodorou and Bowen (1969).

This simple approach has the value that the importance of single factors can be studied under defined conditions. While it falls far short of the complex dynamic situation obtaining in the field, it has been useful in drawing attention to certain factors presenting unsuspected difficulties in establishing mycorrhizal fungi, e.g., soil temperature factors. The eventual aim is to make the system more complex by controlled

introduction of other microorganisms in order to understand better the dynamics of root colonization in soil by mycorrhizal fungi and other microorganisms.

2. Plant Species

Theodorou and Bowen (1971b) found that *R. luteolus* could colonize living roots of the grasses *Lolium perenne* and *Phalaris tuberosa,* the tree species *Eucalyptus leucoxylon* and *E. camaldulensis,* and also the clover *Trifolium subterraneum.* They concluded from this that the absence of mycorrhizal fungi for *P. radiata* from grassland areas distant from forests (in contrast to their presence in many eucalypt stands) (Bowen, 1963) was the nonarrival of spores in sufficient quantity to become established in competition with the native microflora, i.e., the absence from grasslands was probably not due to toxin production by the living grass roots. Their studies did suggest however that *decomposing* grass roots could be toxic to the fungus. Mycelial inoculum was used in these colonization studies, and an alternative interpretation of the failure of mycorrhizal fungi to establish in natural grasslands is that some spores may arrive but the grass roots may not stimulate spore germination. The ability of *R. luteolus* to grow in the rhizosphere of nonhost plants may be important in survival and spread of the fungus in the absence of a suitable mycorrhizal-forming host.

Theodorou and Bowen (1971b) also noted growth of *R. luteolus* was discontinuous along the grass roots, rather than compact and continuous as on pine roots. The detailed reasons for this are unknown.

Claims have been made that certain heath plants (or their associated endomycorrhizal fungi) are toxic to ectomycorrhizal fungi which may be introduced with a tree species, e.g., *Calluna* (Handley, 1963). Colonization experiments similar to those above may help to evaluate such hypotheses, as distinct from effects of plant residues in soil, e.g., Handley (1963).

3. Nutrition

a. pH and Nitrate. Decrease of mycorrhizal production in alkaline soils could be due to a high pH directly or to associated effects, e.g., high nitrification and consequent nitrate depression of mycorrhizal formation as suggested by Richards (1961). Growth of *R. luteolus* in the rhizosphere of *P. radiata* in sterilized forest soil is given in Table VI. The main points to note are (i) the absence of growth in soil and rhizosphere at pH 8 and very low soil nitrate, thus suggesting that poor mycorrhizal formation at alkaline pH may be due as much to inhibition of growth of the fungus in the rhizosphere as to an effect of nitrate on infection and

TABLE VI

EFFECTS OF SOIL pH AND NITRATE CONCENTRATION ON THE COLONIZATION OF ROOTS OF *Pinus radiata* SEEDLINGS AND GLASS FIBERS BY *Rhizopogon luteolus*[a]

pH	Nitrate concn. (ppm)	Number colonized		Av. length colonized (mm)		Fungal growth intensity								Root length per plant (mm)
		Fibers	Seedlings	Fibers	Seedlings	Fibers[b]				Seedlings[b]				
						vh	h	m	l	vh	h	m	l	
6.2	4.5	15 (20)[c]	14 (19)	9.8	16.8	0	3	3	9	7	3	2	2	88.8
5.0	12	11 (20)	19 (20)	4.8	26.5	0	2	0	9	17	2	0	0	83.3
5.0	115	14 (20)	16 (18)	8.5	16.0	0	2	6	6	11	3	2	0	90.2
8.0	12	3 (20)	0 (16)	0.8	0.0	0	0	0	3	—	—	—	—	86.2
8.0	115	5 (20)	0 (5)	1.4	0.0	0	0	1	4	—	—	—	—	65.8
				LSD										LSD
				$P = 0.05$	5.83									$P = 0.05$ 13.9
				$P = 0.01$	7.91									$P = 0.01$ 15.8

[a] From Theodorou and Bowen (1969), by permission of *Aust. J. Bot.*

[b] Very heavy (vh), heavy (h), moderate (m), and light (l).

[c] Numbers in parenthesis represent the number surviving out of 20.

mycorrhizal development. (ii) At pH 5, 115-ppm NO_3–N depressed colonization significantly but had little effect (or the reverse effect) on growth on the glass fiber; thus a possible nitrate effect on plant root exudate is suggested. (iii) The importance of root exudation is shown by the much greater colonization of roots than of fibers. Studies of mycorrhizal formation showed ectendomycorrhizae to completely supplant ectomycorrhizae at pH 8, an experimental demonstration of a prediction by Melin (1953) that different mycorrhizal fungi could be associated with trees under acid and under alkaline conditions.

b. Phosphate. Despite the increased exudation of sugars and amino acids with phosphate deficiency (see Section IV,A,4), we have been unable to show any stimulation of colonization of the rhizosphere of phosphate-deficient *P. radiata* by *R. luteolus*. Perhaps this is not surprising as organic acids in exudates are more abundant than amino acids, and Cartwright (1967) could not detect a phosphorus-deficiency effect on organic acid exudates of *E. pilularis*. We suggest increased mycorrhizal production of pines at relatively low phosphate levels (e.g., Björkman, 1942; Purnell, 1958) are attributable to effects on infection phases and not external root colonization by the fungus.

4. Physical Factors

a. Soil Temperature. Studies on the effect of soil temperature on colonization of *P. radiata* roots (Theodorou and Bowen, 1971a) arose from the realization that the optimum temperatures for growth of mycorrhizal fungi in laboratory media, viz., 18°–29°C (Hacskaylo *et al.*, 1965; Harley, 1969) were frequently well above those recorded at sowing time in many cool temperate climates. Studies on growth of *R. luteolus*, *S. granulatus*, and *S. luteus* in Melin–Norkrans media and the rhizosphere of *P. radiata* with controlled soil temperatures gave the data of Table VII. Statistical ratings of intensity of growth gave similar results to those indicated by the length of root colonized.

With each of the five fungi, the length of root colonized was significantly less at 16°C soil temperature than at 25°C, and, with the exception of *S. granulatus* No. 5, less than at 20°C. In some cases (*S. luteus* No. 1 and *R. luteolus*) there was almost no colonization at 16°C, despite growth of *R. luteolus* at 16°C on laboratory media being half that at 25°C. Predictions of root colonization based on growth in rich laboratory media at 16°C would have been correct with only two of the five fungi studied. This indicates a temperature effect on root metabolism of *P. radiata* (e.g., also Bowen, 1970) and root exudation; since an inordinate reduction occurred with only some fungi, a specific effect rather

TABLE VII

COLONIZATION OF *Pinus radiata* ROOTS AND GROWTH ON MELIN-NORKRANS AGAR BY STRAINS OF MYCORRHIZAL FUNGI AT DIFFERENT TEMPERATURES[a,b]

Fungus	Soil temperature (°C)	Root length (mm)	Length colonized (mm)		Colony diameter in Melin-Norkrans media (mm)[c]
			Seedlings	Fibers	
Suillus luteus No. 1	16	73.4	6.2	4.5	8.9
	20	77.6	15.2	7.4	70.9
	25	82.9	24.9	6.9	63.8
S. luteus No. 3	16	85.6	18.6	5.1	37.7
	20	91.7	29.4	5.1	60.7
	25	92.9	31.8	9.1	61.3
S. granulatus No. 8	16	75.8	16.0	6.1	7.0
	20	90.7	28.0	6.5	41.0
	25	87.1	26.3	8.1	44.8
S. granulatus No. 5	16	90.3	16.8	5.5	63.2
	20	86.7	17.3	7.0	66.7
	25	87.2	24.4	5.5	57.5
Rhizopogon luteolus No. A[d]	16	41.9	4.1	4.4	40.0
	25	60.1	27.3	3.2	82.0
LSD: *Suillus*			5.1	($P = 0.05$)	
			6.7	($P = 0.01$)	
R. luteolus			7.4	($P = 0.01$)	

[a] From Theodorou and Bowen (1971a).

[b] Growth in soil for 4 weeks.

[c] Growth for 28 days, except for *R. luteolus* which was for 11 days.

[d] This experiment was carried out at a different time from that with the species of *Suillus*.

than a reduction in common energy sources at the lower temperature appears to be involved. With *S. granulatus* No. 8 colonization at 16°C was markedly greater than would have been expected from studies in synthetic media. These results indicate the difficulty in extrapolating to growth in the rhizosphere from studies in laboratory media. Hacskaylo *et al.* (1965) showed growth of mycorrhizal fungi at various temperatures is influenced greatly by the laboratory medium, and perhaps therefore a good relationship should not be expected between growth in synthetic media and the vastly different environment of the rhizosphere.

The very poor colonization by some isolates at soil temperatures of 16°C compared with that at 20°C, i.e., as more realistic soil temperatures to those in southern Australian soils at planting are approached, has revealed a possible need to select fungi capable of colonizing roots

under field conditions as well as showing efficiency in plant stimulation. Even within species large differences occurred between isolates in root colonization at 16°C (Table VII, *S. luteus* No. 1 and *S. luteus* No. 3).

We also found better and faster mycorrhizal infection at 20° and 25°C than at 15°C but we do not know if this was due only to better colonization of the root alone or to temperature effects on infection process also. Marx *et al.* (1970) reported ectomycorrhizal development and mycelial growth in pure culture at different temperatures were correlated for isolates of *Thelephora terrestris* but not for *Pisolithus tinctorius.*

b. Soil Moisture. Mycorrhizal fungi differ greatly in their growth at low soil moisture. In laboratory media growth of *R. luteolus* ceases in relative humidities approximating those of soil at wilting point, but *C. graniforme* can grow at much lower relative humidities (Bowen, 1964). Colonization of the rhizosphere of *P. radiata* by *R. luteolus* declines markedly above field capacity and below 50% field capacity (Table VIII), i.e., still in the range of relative humidities supporting growth in laboratory media. Possible differences between fungi in this respect could be a factor in the replacement of some types of mycorrhizae by others in dry soils, reported by Worley and Hacskaylo (1959).

5. *Microbial Factors*

a. Previous Fungal Nutrition. Competition for energy sources in the rhizosphere is likely to decrease the chances of establishment of mycelial forms of inoculum. Using mycelia with high carbohydrate reserves could

TABLE VIII

EFFECT OF SOIL MOISTURE ON COLONIZATION OF ROOTS OF *Pinus radiata* BY *Rhizopogon luteolus*

Moisture level	Length colonized (mm)[a]		Root length[a] (mm)
	Fibers	Seedlings	
25% field capacity	6.6	7.0	101
50% field capacity	10.6	26.0	87
75% field capacity	11.8	22.4	80
Field capacity	10.3	20.0	73
125% field capacity	3.6	0	0
	LSD 4.4 $P = 0.05$		LSD 10 $P = 0.05$
	5.8 $P = 0.01$		13 $P = 0.01$
	7.4 $P = 0.001$		

[a] Mean of 20 plants grown for 4 weeks in sterilized soil. Field capacity was 25% moisture.

enhance growth of the mycorrhizal fungus in the rhizosphere; colonization by hyphae grown in Melin-Norkrans medium with 200 gm/liter glucose was twice that of hyphae grown with 20 gm/liter glucose. Mycorrhizal fungus colonization of the rhizosphere is stimulated by adding as little as 0.2 gm/liter glucose solution to sterile soil (Bowen and Theodorou, 1971); obviously one of the limitations to growth is the energy source in the exudates.

b. Competition and Antagonism. Mycorrhizal fungi are generally considered susceptible to competition from other soil organisms (Harley, 1969) and the successful introduction of mycelial forms of inoculum into fumigated soil (Theodorou, 1967) may be attributed partly to elimination of competitors or antagonists. Evidence exists in laboratory media for antagonism of some fungi to ectomycorrhizal fungi; Brian *et al.* (1945) in investigating a reported toxicity of heath soil to mycorrhizal fungi found that isolates of *Penicillium genseni* produced the antibiotic gliotoxin, to which *S. bovinus*, *S. grevillei* (syn. *B. elegans*), and *Cenococcum graniforme* were very sensitive. Levisohn (1957) reported inhibition of *S. granulatus*, *S. variegatus*, *S. bovinus*, *Leccinum scabrum* (syn. *B. scaber*), and *R. luteolus* by *Alternaria tenuis* in laboratory media. Although care must be taken in extrapolating from such antagonisms in laboratory media to behavior in the field, Levisohn found that *L. scabrum* could be introduced successfully as a mycorrhizal fungus into arable soils in which the other mycorrhizal fungi, which were more markedly affected by *A. tenuis*, failed to grow. Handley (1963) suggested that endomycorrhizal fungi of *Calluna* may be antagonistic toward ectomycorrhizal fungi for coniferous trees planted in heathland soils.

There are few published experimental data on microbial effects on colonization of the rhizosphere by ectomycorrhizal fungi. The easier establishment of mycorrhizal fungi in fumigated soils could be due to possible stimulation of mycorrhizal fungi by microorganisms recolonizing such soils or to elimination of antagonists. Ridge and Theodorou (1972) found that the major bacterial recolonizers of fumigated soils are fluorescent pseudomonads, and we have examined the effect of these and other bacteria on colonization of *R. luteolus* on *P. radiata* roots in sterilized forest soil. The results (Table IX) show a highly significant decrease in root colonization in the presence of pseudomonads, whereas a sporing bacterium with populations as high as pseudomonads had no inhibiting effect. Because of the reduction in colonization of glass fibers also in the presence of pseudomonads, production of antagonistic substances by the pseudomonads appears to be a better interpretation of the results than a simple competition for readily available energy sources

TABLE IX

EFFECT OF BACTERIA ON COLONIZATION OF *Pinus radiata* ROOTS BY *Rhizopogon luteolus*

Treatment	Length colonized (mm)		Root length (mm)
	Seedlings	Fibers	
R. luteolus no bacteria	22.8	9.6	69
R. luteolus + fluorescent *Pseudomonas* R4/F3[a]	10.4	2.4	73
R. luteolus + fluorescent *Pseudomonas* R4/F1[a]	4.0	1.1	87
R. luteolus + *Pseudomonas* R3/AP1[a]	1.2	1.5	80
R. luteolus + sporing bacteria S4/Post 2[a]	19.6	6.0	64
	LSD 3.8 $P = 0.05$		LSD 9 $P = 0.05$
	5.1 $P = 0.01$		11 $P = 0.01$
	6.5 $P = 0.001$		14 $P = 0.001$

[a] Laboratory reference numbers.

in the root exudate. Other studies (Bowen and Theodorou, 1971) have shown a 20–50% reduction in colonization of roots by *R. luteolus* in sterilized soil reinoculated with a general soil microflora. The significant increase in root growth in Table IX with two of the pseudomonads is consistent with findings of both root stimulative and depressive organisms in soil (Bowen and Rovira, 1961).

G. COLONIZATION DYNAMICS

An understanding of the dynamics of the rhizosphere ecosystem in a field soil is necessary to assess the possibility of manipulating the rhizosphere population either for ease of introduction (and for persistence) of mycorrhizal fungi or for reasons such as biological control of plant diseases—of particular value in extensive crops such as forests. The treatment of single factors, as above, especially in a sterile soil is unreal, but it is a first step toward understanding a complex situation and provides quantitative information necessary to building a dynamic picture of the importance of various factors in colonization of roots.

Population biologists in other fields, e.g., zoology, have found the ecosystem or systems analysis approach of considerable use (e.g., Watt, 1968) but it had been largely ignored in the study of microbial colonization of roots. It is not difficult to discern direct analogies between phenomena *thought* to be important in microbial colonization of roots and

population phenomena studied in detail in other fields, e.g., competition, dispersal, food supplies, and stability of systems. At this stage of our knowledge, the predictive value of such approaches in any but very broad terms in rhizosphere biology is small, but it forms a useful framework for integrating concepts of colonization, and particularly for focusing attention on large zones of ignorance which need to be studied.

In primary colonization the root grows through the soil at a rate determined by species and environment and comes into contact with potential bacterial and fungal colonizers of the rhizosphere (possibly including pathogens and propagules of mycorrhizal fungi). Under what conditions will these occupy the new root surface and possibly retard colonization by an inoculated fungus, or will existing colonization determine colonization of younger new root tissue? Actively growing seedling roots of *P. radiata* grow at 3–12 mm/day in well-aerated moist soil between 10° and 25°C (Bowen, 1970) depending on soil conditions, but the rate of growth of hyphae of *R. luteolus* (1 strain), *S. granulatus* (2 strains), and *S. luteus* (2 strains) along the root was of the order of 1–1.5 mm/day at 25°C in the absence of other organisms (Theodorou and Bowen, 1971a) and considerably smaller at lower temperatures. At 25% field capacity, growth of *R. luteolus* along roots was reduced to 0.3 mm/day while that of the root was 5 mm/day. Thus under reasonably good conditions for seedling growth, the root will quickly outgrow a mycorrhizal inoculum of growing hyphae. Where basidiospores must first germinate, the fungus is further delayed. A similar situation occurs with regrowth of dormant roots in the spring when new lateral root growth can temporarily break away from a fungal mantle (Robertson, 1954). Mycelial strands however grow along roots at 2–4 mm/day (at 20°C) (Skinner and Bowen, 1972) and once formed, clearly present a method of fairly rapid colonization of roots. This rapid growth is no doubt due to ready translocation of nutrients from an existing food base. Where root growth slows considerably due to age or adverse conditions, fungal growth may be similar to that of the root, e.g., some basidiomycetes and mycorrhizal fungi, such as *Cenococum graniforme*, can grow at very low soil water contents (Bowen, 1964) and can probably colonize new root growth where this is very slow because of low water content of soil. The complete investment of slowly growing short roots by mycorrhizal fungi thus giving the mycorrhizal mantle is, of course, another example of the fungus keeping up with the root.

A vigorously growing seedling root will easily outgrow mycelial inoculum of mycorrhizal fungi and many purely rhizosphere fungi (Taylor and Parkinson, 1961) leaving large amounts of actively exuding young roots available for colonization. Can microorganisms in soil colonize these before spread of organisms from older parts of root? There is very little

quantitative information available on growth rates of soil bacteria and fungi on different parts of roots. Experiments by Ridge and Rovira (1970) on bacterial spread along roots indicate that in natural soils fresh colonization from soil is much more frequent than colonization from migration of existing organisms along the root when conditions are conducive to rapid root growth. Taylor and Parkinson (1961) considered lateral colonization of new root surfaces from soil to be more usual than colonization by spread of organisms along the root. Colonization of the new root by organisms in soil will depend on the population of the particular organisms in the soil, their distribution and proximity to the root, sensitivity to root exudates, rate of germination, motility to the root when the root does not actually touch them, and growth rate on the root surface. More information is needed on the rate of germination of fungal spores in the rhizosphere but it is known that spores of many soil fungi, e.g., *Penicillium* and *Helminthosporium*, will germinate in 1–10 hours when fungistasis is relieved, e.g., by root exudates, and therefore they are quite capable of rapidly colonizing new root surfaces approaching or touching them, much more quickly than the spread of mycorrhizal and most other fungi along the root. Thus the fungus with many propagules dispersed through soil and capable of germinating rapidly under the influence of the root has an enormous advantage in primary rhizosphere colonization, and it is almost a matter of chance which species of these the root will touch or approach closely to give them the opportunity of colonizing the root. Almost nothing is known of rates of germination of spores of mycorrhizal fungi in soil but it is difficult to envisage them germinating rapidly enough to be among these first colonizers.

It seems certain that mycorrhizal fungi will not be the first colonizers of recently formed seedling roots where growth of these is at all vigorous. What difficulty then does the mycorrhizal fungus (and late arrivals) have in establishing itself? Harley and Waid (1955) have shown that the numbers of fungi isolated from beech roots increase with age and a poorly defined succession occurs. The root tip was often sterile, fast-growing sporing fungi tended to occur toward the tip, and in older parts slow-growing and sometimes sterile fungi occurred as well. How do these late-developing organisms establish?

With herbaceous plants early microbial colonizers, which are predominantly bacteria, cover most of the root surface (Rovira, 1956b). However with other plants, e.g., *P. radiata*, this is not always the case. We have examined colonization of *P. radiata* roots growing in a sandy forest soil by removing seedling roots of known age, gently agitating them in water to remove sand but as few of the microorganisms as possible, and staining them using the Jones and Mollison method (1948).

Direct measurements of the areas of roots covered by microbial growth were made by weighing cut-outs of tracings of bacterial and fungal growth in representative microscopic fields projected onto a screen. We have no way of telling what percentage of organisms were lost in the washing in this study but even if it was 50% in our very gentle washing procedure, the results of Table X are convincing. Usually the percentage of the root surface covered by microorganisms was less than 10%, even with roots 3 weeks old. This was variable and on one occasion 16% was covered in a portion of the root 4 days old. We have not yet examined whether this generally poor colonization was related to a possibly low microbial population of the soil but it seems unlikely; studies with other soils would be desirable. Another possible reason for the low root surface cover by microorganisms is that exudation is localized and exudates are in lower concentration in the uncolonized parts. It is clear that even with relatively old parts of roots in a field soil there can be considerable areas of *P. radiata* root available for colonization and possible unimpeded paths for fungal growth could be discerned. Old sloughed epidermal cells were usually very heavily colonized.

The spatial considerations above raise the question of the extent to which interaction between microorganisms occurs on roots. The results of Table IX show that antagonism or competition for substrates does occur when organisms are added simultaneously in high concentration, and thus there is clearly scope in rhizosphere microbiology, for consideration of competition between species and stability of microecosystems, possibly along similar lines to those proposed for animals by Watt

TABLE X

MICROBIAL OCCUPATION OF *Pinus radiata* RHIZOPLANE SEGMENTS

Plant age (days)	Distance of segment from apex (cm)	Age of segment (days)	Percent occupation of segment[a]
7	2.5	4	1.2
21	14	21	8.4
28	1	1–2	7.1
28	2.5	4	5.4
28	3	4	15.7
28	3.5	4–5	7.2
90	1	1	0
90	4	4	12.1
90	Base	90	36.6

[a] Percentage of root surface covered by microorganisms with seedlings growing in a forest soil.

(1968) and Garfinkel and Sack (1964). However, it is also likely that at least for *P. radiata* in the soil we used, the main phenomenon was a number of fairly small spatially noninteracting communities. There is great scope for detailed studies of microecology and microbial interactions with general soil microorganisms and mycorrhizal fungi, possibly using techniques such as fluorescent antibody reactions (Beutner, 1961; Schmidt and Bankole, 1965; Trinick, 1970).

It seems in many cases (most cases?) late colonizers will establish not because they displace existing microorganisms but because there is space which they can occupy. In doing this, what do the slow-growing fungi and other late arrivals use as energy sources? There are a number of possibilities: (i) They may use exudates (if any) coming from the uncolonized part of the root. (ii) They may use secondary products of the existing microflora. At this time the first colonizers may be actively growing, resting, or senescing—methods used in microbiological examinations do not elucidate the condition of colonies of different ages on the root. Both this possibility and the previous one may be consistent with late colonizers (including mycorrhizal fungi) having lower metabolic rates and lower demands on energy sources than early colonizing, faster growing organisms. Energy requirements both for growth and maintenance of primary colonizers and slower late colonizers in the rhizosphere are unstudied. (iii) They could achieve energy and growth factor supplies by antagonism toward some early colonizers. A number of mycorrhizal fungi have been shown to produce antibiotics in laboratory culture (Šašek and Musílek, 1967; Marx, 1969). Antibiotic production would also help them maintain their colonization of the root against later entries. (iv) They may alter the permeability or metabolism of the host epidermal cells to increase exudation locally—an unproven hypothesis but one with considerable selective advantage. It is possible that antibiotics are involved in this also. (v) They may have access to energy sources not readily available to other organisms—this is a little explored field and somewhat unusual compounds are likely to be involved.

We have considered only energy sources above but the more general rhizosphere environment of mature parts of roots, i.e., including growth factors, inorganic composition, and physical environment, must also be considered in microbial growth on older parts of roots.

H. Growth around Roots following Infection

Following infection of the root, the fungus can obtain its energy and growth sources in an environment free of competition and then translocate them to the external phase. Except where rapid root growth occurs after a dormant period, growth of mycorrhizal fungi can usually proceed

easily in the cortex of long roots of pine (Robertson, 1954; Wilcox, 1968), and therefore growth in the rhizosphere is not so critical for infection of short roots arising from the long root. In other cases, e.g., *Fagus* (Harley, 1969), infection of the long root is less common and external spread will be important. Few quantitative studies have been made on rate of spread of mycorrhizal fungi along the root surface compared with the cortex. Mycelial strands of *R. luteolus* grow along *P. radiata* roots in soil at 15°–20°C at 2–4 mm/day (Skinner and Bowen, 1972).

Growth on the roots following infection is also important when considering pathogen control (Chapter 9), nutrient uptake (Chapter 5), mycorrhizal succession, and spread from tree to tree. Rapid external growth by a mycorrhizal fungus will sometimes enable it to replace an existing mycorrhizal fungus (Marks and Foster, 1967; Wilcox, 1971). Ectendomycorrhizae are usually restricted to young seedlings and are replaced by other types (Mikola, 1965; Wilcox, 1971). These phenomena should be explainable in terms such as growth rate on particular exudates, infection ability of different mycorrhizal fungi, changes in plant susceptibility to infection, competition with other microorganisms, survival from season to season, etc., but there has been little study of such phenomena at this level so far. The spread of fungi from root to root will depend on root contact (especially with fungi where little external mycelium is formed) and the ability of fungi to grow through soils (Robertson, 1954).

V. Conclusions and Future Approaches

Response to mycorrhizal introduction and variation among fungi in their effect on tree growth has been indicated (e.g., Chapter 5) and hence a desirability of selecting fungi for inoculation either to new sites or superimposing them on sites with less beneficial mycorrhizae is apparent. The evidence of this chapter indicates variability among fungi in the ease of introducing them, and selection on this basis and on their ability to persist from season to season as well as on efficiency in tree stimulation is desirable. The rationale for studies of growth of mycorrhizal fungi in the rhizosphere is that an understanding of this will indicate the critical zones for microbial selection and the management practices needed to assist establishment.

Spore inoculation with basidiomycetes has been shown to be an easy practicable way to introduce ectomycorrhizal fungi, and the technology of application has been largely overcome. However this restricts one to the use of fungi with which a ready supply of fruiting bodies is available. Field collection of such sporophores reduces control over inoculum.

More control would be given by the development of methods to induce sporophore formation under laboratory and pilot plant conditions. There is an obvious need also for further study of resistance to adverse conditions and ease of establishment in the field of other types of propagules of mycorrhizal fungi, e.g., oidia, which may be readily produced in laboratory culture. This also imposes some limitation on the fungi which can be used in inoculation and eventually the greater reliability of mycelial forms of inoculum should be aimed at.

Infected roots will generally enable the survival of a fungus from season to season, but studies on survival and spread of different types of propagules under varying soil conditions both in the presence and absence of a host plant will add considerably to our knowledge of persistence of introduced organisms. Development of quick reliable marker techniques for such studies is important. The use of distinctive and reliable morphological markers (see Chapter 2) will be of great use as would be the development of reliable antibody fluorescence techniques for ectomycorrhizal fungi, in a similar way to that used in study of other soil fungi (Schmidt and Bankole, 1965). The development of highly selective media for particular groups of organisms has been of great value in studying microbial ecology (Tsao, 1970; Sands and Rovira, 1970, 1971) and will also be useful in mycorrhizal studies. Similarly, in rhizosphere colonization studies, use could be made of techniques involving labeling of hyphae, e.g., by radioactive isotopes (Robinson and Lucas, 1963).

Growth in the rhizosphere can be considered at two main levels, the descriptive level, where colonization of particular organisms and interactions with other organisms are examined in relation to environmental conditions, and the ecological–physiological level, where the mechanisms of these observations are studied. The study of root exudates and definition of the environment for root growth comes into the second of these categories. Exudates can be viewed from purely plant physiological aspects or attempts can be made to relate them to microbial phenomena on the root surface. Documentation of root exudates in studies performed up to now have provided useful knowledge of some of the main types of substrates in the rhizosphere and the effects of environment on these. They have pointed out, for example, that we should pay more attention to the role of organic acids in nutrition of microorganisms around the root. However, one must ask where do root exudate studies go from here? In the field of pure plant physiology it is obvious that more should be done to relate exudation to the composition of root cells and the mechanisms of loss, and in this way to integrate exudate amount and composition with general plant physiology and known effects of

environment on plant behavior. Biophysical and physiological approaches to nutrient uptake and loss mechanisms and compartmentation in cells are fundamentally important in such studies of root exudation. Detailed root exudation analyses on some major groups of compounds would now appear to be sufficient to provide a realistic background for many rhizosphere microbiology studies. Some other groups of compounds need much more study, e.g., volatiles, to lay a similar type of background there. Further detailed analyses of sugars, organic acids, and amino acids should be related to specific realistic questions, e.g., exudation of citric acid for its role in desorption of phosphate from soils or for fluctuation in key compounds known to be important in the ecology of a particular microorganism. Further pure data collections without specific questions on the commonly occurring compounds seem unwarranted. Occurrence of compounds likely to be used by a wide range of microorganisms is not likely to answer questions of microbial specificity—the more unusual compounds would be more likely to do that. An illustration of this point was given by Gunner *et al.* (1966) who found that the dominant bacterium from roots of bean plants given foliar applications of the organo phosphate insecticide "diazinon" used this as a source of sulfur, phosphorus, carbon, and nitrogen. It may be possible, similarly, to stimulate the secretion of specific energy sources (if such exist) for selected mycorrhizal fungi in the rhizosphere by foliar application of the energy source.

In relating root exudates to microbial growth around roots, not only must specificity in microbial response be considered but also the important question is whether certain microorganisms themselves selectively enhance exudation—such a property by some mycorrhizal fungi could enhance their establishment in a highly competitive environment.

There are tremendous gaps to be filled in understanding microbial colonization of roots in such matters as the dynamics of growth on surfaces, the dynamics of competition, energy relations of organisms for growth and maintenance, production of antibiotics under rhizosphere conditions, definition of ecological "niches" on roots, quantitative studies on growth rates at different parts of roots under different environmental conditions, migration rates along roots, and germination of soil propagules and establishment on the root. Growth of mycorrhizal fungi on the young root must be integrated with this knowledge. A really quantitative comprehensive understanding of microbial colonization of roots in natural soils is not attainable at the moment. However, (i) since so many organisms have the ability to colonize roots and whichever do so is very much a chance occurrence depending on placement of propagules in soil, and (ii) since it seems probable that many spatially separated partially independent communities could be involved, it is doubtful if a very detailed predictive statement of microbial composition

at all parts of a root under various conditions is ever likely to be possible. It is more realistic to use information gained by such studies as those above to help specify the general characters of microorganisms one should select for ready colonization, persistence, and spread. Certainly it would seem that one has a greater chance of partially controlling the rhizosphere microflora of plants by the use of organisms which also infect the roots, e.g., mycorrhizal fungi, but first the infection must be established.

In this chapter we have not considered the mechanisms of replacement of one mycorrhizal fungus with another, and superimposition of selected mycorrhizal fungi on existing, possibly less desirable ones. This important problem awaits further study.

References

Agnihotri, V. P., and Vaartaja, O. (1967). Root exudates from red pine seedlings and their effects on *Pythium ultimum*. *Can. J. Bot.* **45**, 1031.

Barber, S. A. (1962). A diffusion and mass-flow concept of soil nutrient availability. *Soil Sci.* **93**, 39.

Barber, S. A., and Ozanne, P. G. (1970). Autoradiographic evidence for the differential effect of four plant species in altering the calcium content of the rhizosphere soil. *Soil Sci. Soc. Amer., Proc.* **34**, 635.

Barber, S. A., Walker, J. M., and Vasey, E. H. (1963). Mechanisms for the movement of plant nutrients from the soil and fertilizer to the plant root. *J. Agr. Food Chem.* **11**, 204.

Beutner, H. E. (1961). Immuno-fluorescent staining—the fluorescent antibody method. *Bacteriol. Rev.* **25**, 49.

Björkman, E. (1942). Uber die Bedingungen der Mykorrhiza bildung bei Kiefer und Fichte. *Symb. Bot. Upsal.* **6**, No. 2, 1.

Boulter, D., Jeremy, J. J., and Wilding, M. (1966). Amino acids liberated into the culture medium by pea seedling roots. *Plant. Soil* **24**, 121.

Bowen, G. D. (1961). The toxicity of legume seed diffusates toward rhizobia and other bacteria. *Plant Soil* **15**, 155.

Bowen, G. D. (1963). The natural occurrence of mycorrhizal fungi for *Pinus radiata* in South Australian soils. *CSIRO Div. Soils, Adelaide, Aust., Div. Rep.* **6/63**.

Bowen, G. D. (1968). Chloride efflux along *Pinus radiata* roots. *Nature (London)* **218**, 686.

Bowen, G. D. (1969). Nutrient status effects on loss of amides and amino acids from pine roots. *Plant Soil* **30**, 139.

Bowen, G. D. (1970). Effects of soil temperature on root growth and on phosphate uptake along *Pinus radiata* roots. *Aust. J. Soil. Res.* **8**, 31.

Bowen, G. D. (1964). Unpublished data.

Bowen, G. D., and Kennedy, M. (1959). Effect of high soil temperatures on *Rhizobium* spp. *Queensl. J. Agr. Sci.* **16**, 177.

Bowen, G. D., and Rovira, A. D. (1961). The effects of micro-organisms on plant growth. I. Development of roots and root hairs in sand and agar. *Plant Soil* **15**, 166.

Bowen, G. D., and Rovira, A. D. (1969). The influence of micro-organisms on growth and metabolism of plant roots. *In* "Root Growth" (W. J. Whittington, ed.), pp. 170–201. Butterworth, London.

Bowen, G. D., and Theodorou, C. (1971). Unpublished data.

Brian, P. W., Hemming, H. G., and McGowan, J. C. (1945). Origin of a toxicity to mycorrhiza in Wareham Heath. *Nature (London)* **155**, 637.

Briggs, G. E., Hope, A. B., and Robertson, R. N. (1961). "Electrolytes and Plant Cells." Blackwell, Oxford.

Cartwright, J. B. (1967). A study of the interaction between *Cylindrocarpon radicicola* Wr. and the roots of blackbutt (*Eucalyptus pilularis* Sm.). Ph.D. Thesis, University of Sydney.

Chilvers, G. A., and Pryor, L. D. (1965). The structure of Eucalypt mycorrhizas. *Aust. J. Bot.* **13**, 245.

Cirillo, V. P. (1961). Sugar transport in micro-organisms. *Annu. Rev. Microbiol.* **15**, 197.

Dobbs, C. G., and Hinson, W. H. (1953). A widespread fungistasis in soils. *Nature (London)* **172**, 197.

Ferenczy, L. (1956). Occurrence of antibacterial compounds in seeds and fruits. *Acta Biol. (Budapest)* **6**, 317.

Foster, R. C., and Marks, G. C. (1967). Observations on the mycorrhizas of forest trees. II. The rhizosphere of *Pinus radiata* D. Don. *Aust. J. Biol. Sci.* **20**, 915.

Frenzel, B. (1960). Zur Ätiologie der Anreicherung von Aminosäuren und Amiden im Wurzelraum von *Helianthus annus* L.: Ein Beitrag zur Klärung der Probleme der Rhizosphäre. *Planta* **55**, 169.

Fries, N. (1943). Untersuchungen über Sporenkeimung und Mycelentwicklung bodenbewohnender Hymenomyceten. *Symb. Bot. Upsal.* **6**, No. 4, 1.

Fries, N. (1966). Chemical factors in the germination of spores of Basidiomycetes. *In* "The Fungus Spore" (M. F. Madelin, ed.), pp. 189–99. Butterworth, London.

Fries, N., and Forsman, B. (1951). Quantitative determination of certain nucleic acid derivatives in pea root exudate. *Physiol. Plant.* **4**, 410.

Garfinkel, D., and Sack, R. (1964). Digital computer simulation of an ecological system, based on a modified mass action law. *Ecology* **45**, 502.

Garrett, S. D. (1970). "Pathogenic Root-Infecting Fungi." Cambridge Univ. Press, London and New York.

Greenwood, D. J. (1969). Effect of oxygen distribution in soil on plant growth. *In* "Root Growth" (W. J. Whittington, ed.), pp. 202–223. Butterworth, London.

Gunner, A. B., Zuckerman, B. M., Walker, R. W., Miller, C. W., Deubert, K. H., and Longley, Ruth E. (1966). The distribution and persistence of Diazonin applied to plant and soil and its influence on rhizosphere and soil microflora. *Plant Soil* **25**, 249.

Hacskaylo, E., Palmer, J. G., and Vozzo, J. A. (1965). Effect of temperature on growth and respiration of ectotrophic mycorrhizal fungi. *Mycologia* **57**, 748.

Handley, W. R. C. (1963). Mycorrhizal associations and *Calluna* heathland afforestation. *Forest. Commun. Bull.* **36**.

Harley, J. L. (1969). "The Biology of Mycorrhiza." Leonard Hill, London.

Harley, J. L., and Waid, J. S. (1955). A method of studying active mycelia on living roots and other surfaces in the soil. *Plant Soil* **7**, 96.

Harmsen, G. W., and Jager, G. (1963). Determination of the quantity of carbon and nitrogen in the rhizosphere of young plants. *In* "Soil Organisms" (J.

Doeksen and J. van der Drift, eds.), pp. 245–51. North-Holland Publ., Amsterdam.

Highinbotham, N. (1968). Cell electropotential and ion transport in higher plants. *In* "Transport and Distribution of Matter in Cells of Higher Plants" (K. Mothes *et al.*, eds.), pp. 167–77. Akademie-Verlag, Berlin.

Hiltner, L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache. *Arb. Deut. Landwirtschaftsges.* **98**, 59.

Jenny, H., and Grossenbacher, K. (1963). Root-soil boundary zones as seen in the electron microscope. *Soil Sci. Soc. Amer., Proc.* **27**, 273.

Jones, P. C. T., and Mollison, J. E. (1948). A technique for the quantitative estimation of soil micro-organisms. *J. Gen. Microbiol.* **2**, 54.

Katznelson, H., Rouatt, J. W., and Peterson, E. A. (1962). The rhizosphere effect of mycorrhizal and non-mycorrhizal roots of yellow birch seedlings. *Can. J. Bot.* **40**, 377.

Krupa, S., and Fries, N. (1971). Studies on ectomycorrhizae of pine. I. Production of volatile compounds. *Can. J. Bot.* **49**, 1425.

Laiho, O., and Mikola, P. (1964). Studies on the effect of some eradicants on mycorrhizal development in forest nurseries. *Acta Forest. Fenn.* **77**, 1.

Lamb, R. J., and Richards, B. N. (1971a). Effect of mycorrhizal fungi on the growth and nutrient status of slash and Radiata pine seedlings. *Aust. Forest.* **35**, 1.

Lamb, R. J., and Richards, B. N. (1971b). Unpublished data.

Levisohn, I. (1957). Antagonistic effects of *Alternaria tenuis* on certain root-fungi of forest trees. *Nature (London)* **179**, 1143.

Levisohn, I. (1960). Root forking of pine seedlings growing under non-sterile conditions. *New Phytol.* **59**, 326.

Lewis, D. G., and Quirk, J. P. (1967). Phosphate diffusion in soil and uptake by plants. III. ^{31}P movement and uptake by plants as indicated by ^{32}P autoradiography. *Plant Soil* **26**, 445.

Lewis, D. H., and Harley, J. L. (1965a). Carbohydrate physiology of mycorrhizal roots of beech. I. Identity of endogenous sugars and utilization of exogenous sugars. *New Phytol.* **64**, 224.

Lewis, D. H., and Harley, J. L. (1965b). Carbohydrate physiology of mycorrhizal roots of beech. II. Utilization of exogenous sugars by uninfected and mycorrhizal roots. *New Phytol.* **64**, 238.

Lewis, D. H., and Harley, J. L. (1965c). Carbohydrate physiology of mycorrhizal roots of beech. III. Movement of sugars between host and fungus. *New Phytol.* **64**, 256.

Lockwood, J. L. (1964). Soil fungistasis. *Annu. Rev. Phytopathol.* **2**, 341.

Lundeberg, G. (1970). Utilisation of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi. *Stud. Forest. Suec.* **79**, 1.

MacDougall, B. M. (1968). The exudation of C^{14}-labelled substances from roots of wheat seedlings. *Trans. Int. Congr. Soil Sci., 9th, 1968*, Vol. 3, p. 647.

MacDougall, B. M. (1970). Movement of ^{14}C-photosynthate into the roots of wheat seedlings and exudation of ^{14}C from intact roots. *New Phytol.* **69**, 37.

MacDougall, B. M., and Rovira, A. D. (1965). Carbon-14 labelled photosynthate in wheat root exudates. *Nature (London)* **207**, 1104.

MacDougall, B. M., and Rovira, A. D. (1970). Sites of exudation of ^{14}C-labelled compounds from wheat roots. *New Phytol.* **69**, 999.

Marks, G. C., and Foster, R. C. (1967). Succession of mycorrhizal associations or individual roots of radiata pine. *Aust. Forest.* **31**, 193.

Martin, J. K. (1971). ^{14}C-labelled material leached from the rhizosphere of plants supplied with $^{14}CO_2$. *Aust. J. Biol. Sci.* **24**, 1131.

Marx, D. H. (1969). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. II. Production, identification, and biological activity of antibiotics produced by *Leucopaxillus cerealis* var. *piceina*. *Phytopathology* **59**, 411.

Marx, D. H., and Bryan, W. C. (1969). Studies on ectomycorrhizae of pine in an electronically air filtered, air-conditioned, plant growth room. *Can. J. Bot.* **47**, 1903.

Marx, D. H., and Ross, E. W. (1970). Aseptic synthesis of ectomycorrhizae on *Pinus taeda* by basidiospores of *Thelephora terrestris*. *Can. J. Bot.* **48**, 197.

Marx, D. H., Bryan, W. C., and Davey, C. B. (1970). Influence of temperature on aseptic synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on loblolly pine. *Forest Sci.* **16**, 424.

Melin, E. (1925). "Untersuchungen über die Bedeutung der Baummykorrhiza. Eine ökologische physiologische Studie." Fisher, Jena.

Melin, E. (1953). Mycorrhizal relations in plants. *Annu. Rev. Plant Physiol.* **4**, 325.

Melin, E. (1959). Physiological aspects of mycorrhizae of forest trees. *In* "Tree Growth" (T. T. Kozlowski, ed), pp. 247–63. Ronald Press, New York.

Melin, E. (1963). Some effects of forest tree roots on mycorrhizal basidiomycetes. *Symp. Soc. Gen. Microbiol.* **13**, 125–45.

Melin, E., and Krupa, S. (1971). Studies on ectomycorrhizae of pine. II. Growth inhibition of mycorrhizal fungi by volatile organic constituents of *Pinus sylvestris* (Scots pine) roots. *Physiol. Plant.* **25**, 337.

Mikola, P. (1965). Studies on the ectendotrophic mycorrhiza of pine. *Acta Forest. Fenn.* **79**, 1.

Mitchell, P. (1951). Physical factors affecting growth and death. *In* "Bacterial Physiology" (C. H. Werkman and P. W. Wilson, eds.), pp. 126–77. Academic Press, New York.

Moodie, C. D. (1965). Sites of nutrient exchange in soils. *In* "Microbiology and Soil Fertility" (C. M. Gilmour and O. N. Allen, eds.), pp. 1–16. Oregon State Univ. Press, Corvallis.

Moser, M. (1958). Der Einfluss tiefer Temperaturen auf das Wachstum und die Lebenstätigkeit höherer Pitze mit spezieller Berücksichtigung von Mykorrhizapilzen. *Sydowia* **12**, 386.

Neal, J. L., Bollen, W. B., and Zak, B. (1964). Rhizosphere microflora associated with mycorrhizae of Douglas Fir. *Can. J. Microbiol.* **10**, 259.

Neumann, R. (1959). Relationships between *Pisolithus tinctorius* (Mich. ex. Pers) Coker et Couch. and *Eucalyptus camaldulensis* Dehn. *Bull. Res. Counc. Isr., Sect. D* **7**, 116.

Nightingale, G. T. (1934). Ammonium and nitrate nutrition of dormant Delicious apple trees at 48°F. *Bot. Gaz. (Chicago)* **95**, 437.

Norman, A. G. (1955). The effect of polymyxin on plant roots. *Arch. Biochem. Biophys.* **58**, 461.

Norman, A. G. (1961). Microbial products affecting root development. *Trans. Int. Congr. Soil Sci., 7th, 1960* Vol. 2, p. 531.

Oswald, E. T., and Ferchau, H. A. (1968). Bacterial associations of coniferous mycorrhizae. *Plant Soil* **28**, 187.

Palmer, J. G., and Hacskaylo, E. (1970). Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. *Physiol. Plant.* **23**, 1187.

Pearson, R., and Parkinson, D., (1961). The sites of excretion of ninhydrin-positive substances by broad bean seedlings. *Plant Soil* **13**, 391.

Picci, G. (1959). Dossagio microbiologico di alcune vitamine e di alcuni aminoacidi ceduti dal seme durante la germinazione. *Ann. Fac. Agr. Univ. Pisa* **20**, 51.

Purnell, H. M. (1958). Nutritional studies of *Pinus radiata* D. Don. I. Symptoms due to deficiency of some major elements. *Aust. Forest.* **22**, 82.

Ramdas, L. A., and Dravid, R. K. (1936). Soil temperatures in relation to other factors controlling the dispersal of solar radiation at the earth's surface. *Proc. Nat. Inst. Sci. India* **2**, 131.

Richards, B. N. (1961). Soil pH and mycorrhiza development in *Pinus*. *Nature (London)* **190**, 105.

Richter, M., Wilms, W., and Scheffer, F. (1968). Determination of root exudates in a sterile continuous flow culture. I. The culture method. *Plant Physiol.* **43**, 1741.

Ridge, E. H., and Rovira, A. D. (1970). Unpublished data.

Ridge, E. H., and Theodorou, C. (1972). The effect of soil fumigation on microbial recolonization and mycorrhizal infection. *Soil Biol. Biochem.* **4**, 295.

Riley, D., and Barber, S. A. (1969). Bicarbonate accumulation and pH changes at the soybean root-soil interface. *Soil Sci. Soc. Amer., Proc.* **33**, 905.

Riley, D., and Barber, S. A. (1970). Salt accumulation at the soybean root-soil interface. *Soil Sci. Soc. Amer., Proc.* **34**, 154.

Robertson, N. F. (1954). Studies on the mycorrhiza of *Pinus sylvestris*. I. The pattern of development of mycorrhizal roots and its significance for experimental studies. *New Phytol.* **53**, 253.

Robinson, R. K., and Lucas, R. L. (1963). The use of isotopically labelled mycelium to investigate the host range and rate of spread of *Ophiobolus graminis*. *New Phytol.* **62**, 50.

Rovira, A. D. (1956a). Plant root excretions in relation to the rhizosphere effect. I. Nature of root exudate from oats and peas. *Plant Soil* **7**, 178.

Rovira, A. D. (1956b). Study of the development of the root surface microflora during the initial stages of plant growth. *J. Appl. Bacteriol.* **19**, 72.

Rovira, A. D. (1965). Plant root exudates and their influence upon soil microorganisms. *In* "Ecology of Soil-borne Plant Pathogens" (K. F. Baker and W. C. Snyder, eds.), Vol. I, pp. 170–86. Univ. of California Press, Berkeley.

Rovira, A. D. (1969a). Plant root exudates. *Bot. Rev.* **35**, 35.

Rovira, A. D. (1969b). Diffusion of carbon compounds away from wheat roots. *Aust. J. Biol. Sci.* **22**, 1287.

Rovira, A. D., and Bowen, G. D. (1970). Translocation and loss of phosphate along roots of wheat seedlings. *Planta* **93**, 15.

Rovira, A. D., and Davey, C. B. (1972). Biology of the rhizosphere. *In* "The Plant Root and its Environment." Southern Reg. Educa. Bd. Inst., Virginia (in press).

Rovira, A. D., and MacDougall, B. M. (1967). Microbiological and biochemical aspects of the rhizosphere. *In* "Soil Biochemistry" (A. D. McLaren and G. H. Peterson, eds.), pp. 418–63. Dekker, New York.

Sands, D. C., and Rovira, A. D. (1970). Isolation of fluorescent pseudomonads with a selective medium. *Appl. Microbiol.* **20**, 513.

Sands, D. C., and Rovira, A. D. (1971). *Pseudomonas fluorescens* Biotype G., the dominant fluorescent pseudomonad in South Australian soils and wheat rhizospheres. *J. Appl. Bacteriol.* **34**, 261.

Šašek, V., and Musílek, V. (1967). Cultivation and antibiotic activity of mycorrhizal basidiomycetes. *Folia Microbiol. (Prague)* **12**, 515.

Schmidt, E. L., and Bankole, R. O. (1965). Specificity of immunofluorescent staining for study of *Aspergillus flavus* in soil. *Appl. Microbiol.* **13**, 673.

Skinner, M. F., and Bowen, G. D. (1972). Unpublished data.

Slankis, V., Runeckles, V. C., and Krotkov, G. (1964). Metabolites liberated by roots of white pine (*Pinus strobus L.*) seedlings. *Physiol. Plant.* **17**, 301.

Smith, D., Muscatine, L., and Lewis, D. (1969). Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biol. Rev.* **44**, 17.

Smith, W. H. (1969). Release of organic materials from the roots of tree seedlings. *Forest Sci.* **15**, 138.

Smith, W. H. (1970). Root exudates of seedling and mature sugar maple. *Phytopathology* **60**, 701.

Subba-Rao, M. S., Bidwell, R. G. S., and Bailey, D. L. (1962). Studies of rhizosphere activity by the use of isotopically labelled carbon. *Can. J. Bot.* **40**, 203.

Taylor, G. S., and Parkinson, D. (1961). The growth of saprophytic fungi on root surfaces. *Plant Soil* **15**, 261.

Theodorou, C. (1967). Inoculation with pure cultures mycorrhizal fungi of radiata pine growing in partially sterilized soil. *Aust. Forest.* **31**, 303.

Theodorou, C. (1971). Introduction of mycorrhizal fungi into soil by spore inoculation of seed. *Aust. Forest.* **35**, 23.

Theodorou, C., and Bowen, G. D. (1968). Unpublished data.

Theodorou, C., and Bowen, G. D. (1969). The influence of pH and nitrate on mycorrhizal associations of *Pinus radiata* D. Don. *Aust. J. Bot.* **17**, 59.

Theodorou, C., and Bowen, G. D. (1970). Mycorrhizal responses of radiata pine in experiments with different fungi. *Aust. Forest.* **34**, 183.

Theodorou, C., and Bowen, G. D. (1971a). Influence of temperature on the mycorrhizal associations of *Pinus radiata* D. Don. *Aust. J. Bot.* **19**, 13.

Theodorou, C., and Bowen, G. D. (1971b). Effects of non-host plants on growth of mycorrhizal fungi of radiata pine. *Aust. Forest.* **35**, 17.

Theodorou, C., and Bowen, G. D. (1973). Inoculation of seeds and soil with basidiospores of mycorrhizal fungi. *Soil Biol. Biochem.* — submitted.

Trinick, M. J. (1970). Rhizobium interactions with soil micro-organisms. Ph.D. Thesis, University of Western Australia, Perth.

Tsao, P. H. (1970). Selective media for isolation of pathogenic fungi. *Annu. Rev. Phytopathol.* **8**, 157.

Warcup, J. H. (1967). Fungi in soil. *In* "Soil Biology" (N. A. Burgess and F. Raw, eds.), pp. 51–110. Academic Press, New York.

Watt, K. E. F. (1968). "Ecology and Resource Management." McGraw-Hill, New York.

Wilcox, H. E. (1968). Morphological studies of the roots of Red pine, *Pinus resinosa*. II. Fungal colonisation of roots and the development of mycorrhizae. *Amer. J. Bot.* **55**, 686.

Wilcox, H. E. (1971). Morphology of ectendomycorrhizae in *Pinus resinosa*. *In* "Mycorrhizae" (E. Hacskaylo, ed.), USDA Forest Serv. Misc. Publ. No. 1189, pp. 54–68. US Govt. Printing Office, Washington, D. C.

Wilde, S. A. (1946). "Forest Soils and Forest Growth." Chronica Botanica, Waltham, Massachusetts.

Worley, J. F., and Hacskaylo, E. (1959). The effect of available soil moisture on the mycorrhizal association of Virginia pine. *Forest Sci.* **5**, 267.